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(71) Applicant (for all designated States except US): BAYER AKTIENGESELLSCHAFT [DE/DE]; 51368 Leverkusen (DE).

(72) Inventor; and
(75) Inventor/Applicant (for US only): RAMAKRISHNAN, Shyam [IN/US]; 76 Easton Road, Apt. #10, Brighton, MA 02135 (US).

(74) Common Representative: BAYER AKTIENGESELLSCHAFT; 51368 Leverkusen (DE).

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(54) Title: REGULATION OF HUMAN 11 BETA-HYDROXYSTEROID DEHYDROGENASE 1-LIKE ENZYME

(57) Abstract: Reagents which regulate human 11 beta-hydroxysteroid dehydrogenase 1-like enzyme and reagents which bind to human 11 beta-hydroxysteroid dehydrogenase 1-like enzyme gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to CNS disorders, osteoporosis, liver disease, obesity, blood pressure or fetal development abnormalities, and diabetes.

WO 02/02797 A2

REGULATION OF HUMAN 11 BETA-HYDROXYSTEROID DEHYDROGENASE 1-LIKE ENZYME

5   ## TECHNICAL FIELD OF THE INVENTION

The invention relates to the regulation of human 11 beta-hydroxysteroid dehydrogenase 1-like enzyme.

10   ## BACKGROUND OF THE INVENTION

Glucocorticoid levels in tissues are modulated by the enzyme 11ß-hydroxysteroid dehydrogenase. Two isoforms of the enzyme have been isolated. The type I enzyme (11ß-HSD1) is a bifunctional enzyme which acts predominantly as an oxoreductase to
15   form the active glucocorticoids cortisol (in man) or corticosterone (in rodents) from their inactive 11-keto metabolites, cortisone and 11-dehydrocorticosterone, respectively. Agarwal et al., J. Biol. Chem 264, 18939-46, 1989. The type II isoform (11ß-HSD2) acts unindirectionally to produce inactive 11-keto metabolites. It utilizes NAD to metabolize glucocorticoids to 11-keto compounds with low affinity for gluco-
20   corticoid and mineralocorticoid receptors. Albiston et al., Molec. Cell Endocr 105, R11-17, 1994; Funder et al., Science 242, 583-85, 1988; Edwards et al., Lancet 2, 986-89, 1988.

Gene deletion experiments in mice indicate that this enzyme is important for the
25   maintenance of normal serum glucocorticoid levels and is involved in the activation of key hepatic gluconeogenic enzymes. Other important sites of action include omental fat, ovary, kidney, brain, and vasculature. Koozowsky et al., J. Steroid Biochem. Mol. Biol. 69, 391-401, 1999. 11ß-HSD1 activity is found in all major blood vessels and the heart and is higher in resistance vessels, suggesting a role of 11ß-HSD1 in modu-
30   lating blood pressure. Walker et al, Endocrinol. 129, 3305-12, 1991. 11ß-HSD1 and 11ß-HSD2 mRNA coexist in rat aortic endothelial cells, with the 11ß-HSD1 isoform

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predominating. Brem *et al.*, *Hypertension* 31, 459-62, 1998. Selective inhibition of 11ß-HSD1 activity attenuates the contractile effect of phenylephrine and angiotensin II on aortic rings. Brem *et al.*, *Hypertension* 30, 449-54, 1997.

5   The highest concentrations of 11ß-HSD1 enzyme are in the liver. Immunohisto-chemical studies in human liver show that 11ß-HSD1 is concentrated around the cen-tral vein. Ricketts *et al.*, *J. Endocrinol.* 156, 159-68, 1998. Hepatic 11ß-HSD1 par-ticipates in detoxification mechanisms. Opperman *et al.*, *Eur. J. Biochem.* 227, 202-08, 1995. The levels of the two 11ß-HSD1 isoforms differ and change during fetal
10   brain development. High amounts of 11ß-HSD1 are found in rat kidneys and in hu-man placenta, but lower levels are seen in women taking oral contraceptives. See review by Kozowsky, *Mol. Cell. Endocrin.* 101, 121-27, 1999.

In the rat liver, 11ß-HSD1 mRNA is present at low levels in the neonate, increasing to
15   a plateau at 8 weeks of age (Krozowski *et al.*, 1990). Males have about 20-fold more 11ß-HSD1 mRNA than females, and the levels in females are suppressed by estradiol and growth hormone. Low *et al.*, *J. Endocrinol.* 143, 541-48, 1994; Albiston *et al.*, *Mol. Cell. Endocrinol.* 109, 183-88, 1995.

20   Factors that affect 11ß-HSD1 expression are known. In the hepatoma cell line 2S FAZA, 11ß-HSD1 activity and mRNA expression are increased by dexamethasone and decreased by forskolin and insulin-like growth factor 1. Voice *et al.*, *Biochem J.* 317, 621-25, 1996. Studies in rat and human hepatocytes have shown that thyroid hormone and progesterone exert species-specific effects on 11ß-HSD1 activity but
25   have no effect on message levels. Ricketts *et al.*, *J. Clin. Endocrinol. Metab.* 83, 1325-35, 1998. Exposure of rats to dexamethasone in late pregnancy results in sig-nificant increases in glucocorticoid receptor and phosphoenolpyruvate carboxykinase, without changes in 11ß-HSD1 in the adult offspring. Nyirenda *et al.*, *J. Clin. Invest.* 101, 2174-81, 1998.

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It is obvious that 11ß-HSD1 expression and its regulation are delicately balanced, as well as tissue- and developmental-stage specific. A need exists for further identification of similar enzymes which can be regulated to provide therapeutic effects, for example, via developmental stage- or tissue-specific agonists/antagonists.

5

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a human 11ß-HSD1-like enzyme. This and other objects of the invention are provided by one

10   or more of the embodiments described below.

One embodiment of the invention is a 11β-HSD1-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:

15   amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

20   Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a 11β-HSD1-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:

25   amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

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Binding between the test compound and the 11β-HSD1-like enzyme polypeptide is detected. A test compound which binds to the 11β-HSD1-like enzyme polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the activity of the 11β-HSD1-like enzyme.

5

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a 11β-HSD1-like enzyme polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

10

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

15

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the 11β-HSD1-like enzyme through interacting with the 11β-HSD1-like enzyme mRNA.

20

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a 11β-HSD1-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:

25

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

30

A 11β-HSD1-like enzyme activity of the polypeptide is detected. A test compound which increases 11β-HSD1-like enzyme activity of the polypeptide relative to 11β-HSD1-like enzyme activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases 11β-HSD1-like enzyme activity of the polypeptide relative to 11β-HSD1-like enzyme activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a 11β-HSD1-like enzyme product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

Binding of the test compound to the 11β-HSD1-like enzyme product is detected. A test compound which binds to the 11β-HSD1-like enzyme product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a 11β-HSD1-like enzyme polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

11β-HSD1-like enzyme activity in the cell is thereby decreased.

5   The invention thus provides a human 11ß-HSD1-like enzyme which can be used to identify test compounds which may act, for example, as agonists or antagonists at the enzyme's active site. Human 11ß-HSD1-like enzyme and fragments thereof also are useful in raising specific antibodies which can block the enzyme and effectively reduce its activity.

10

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1   shows the DNA-sequence encoding a 11β-HSD1-like enzyme polypeptide (SEQ ID NO:1).

15   Fig. 2   shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO:2).

Fig. 3   shows the amino acid sequence of the protein identified with SwissProt Accession No. P16232 (SEQ ID NO:3).

Fig. 4   shows the DNA-sequence encoding a 11β-HSD1-like enzyme polypeptide

20   (SEQ ID NO:4).

Fig. 5   shows the DNA-sequence encoding a 11β-HSD1-like enzyme polypeptide (SEQ ID NO:5).

Fig. 6   shows the DNA-sequence encoding a 11β-HSD1-like enzyme polypeptide (SEQ ID NO:6).

25   Fig. 7   shows the BLASTX alignment of 11ß-HSD1-like enzyme polypeptide (SEQ ID NO:2) with the protein identified with SwissProt Accession No. P16232 (SEQ ID NO:3).

DETAILED DESCRIPTION OF THE INVENTION

30

The invention relates to an isolated polynucleotide encoding a 11ß-HSD1-like enzyme polypeptide and being selected from the group consisting of:

a)   a polynucleotide encoding a 11ß-HSD1-like enzyme polypeptide comprising
5   an amino acid sequence selected from the group consisting of:
     amino acid sequences which are at least about 50% identical to
     the amino acid sequence shown in SEQ ID NO: 2; and
     the amino acid sequence shown in SEQ ID NO: 2.

b)   a polynucleotide comprising the sequence of SEQ ID NO: 1;

10   c)   a polynucleotide which hybridizes under stringent conditions to a polynucleo-
     tide specified in (a) and (b);

d)   a polynucleotide the sequence of which deviates from the polynucleotide se-
     quences specified in (a) to (c) due to the degeneration of the genetic code; and

e)   a polynucleotide which represents a fragment, derivative or allelic variation of
15   a polynucleotide sequence specified in (a) to (d).

Furthermore, it has been discovered by the present applicant that a novel 11ß-HSD1-like enzyme, particularly a human 11ß-HSD1-like enzyme, is a discovery of the present invention. Human 11ß-HSD1-like enzyme comprises the amino acid sequence shown in SEQ ID NO:2 (GenBank Accession No. AW163684). Human 11ß-HSD1-like enzyme was identified by searching human sequences with a rat corticosteroid 11ß dehydrogenase (SEQ ID NO:3, SwissProt Accession No. P16232). Human 11ß-HSD1-like enzyme is 43% identical over 86 amino acids to the rat protein identified with SwissProt Accession No. P16232 (FIG. 7). The human 11ß-HSD1-like enzyme of the invention is therefore expected to be useful for the same purposes as previously identified hydroxysteroid dehydrogenases, such as the careful balancing of steroid levels in various tissues and during fetal development. Further, steroid regulation is expected to have a significant role in osteoporosis. Thus, human 11ß-HSD1-like enzyme can be used in therapeutic methods to treat disorders such as CNS, osteoporosis, blood pressure, fetal development disorders, and diabetes. Human 11ß-HSD1-like

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enzyme also can be used to screen for human 11ß-HSD1-like enzyme agonists and antagonists and in diagnostic methods.

Polypeptides

5

11ß-HSD1-like polypeptides according to the invention comprise at least 14, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 125, 200, or 220 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:2 or a biologically active variant thereof, as defined below. An 11ß-HSD1-like polypeptide of the invention

10   therefore can be a portion of an 11ß-HSD1-like enzyme protein, a full-length 11ß-HSD1-like enzyme protein, or a fusion protein comprising all or a portion of an 11ß-HSD1-like enzyme protein.

Biologically Active Variants

15

11ß-HSD1-like polypeptide variants which are biologically active, *i.e.*, retain the ability to interconvert cortisol and cortisone, also are 11ß-HSD1-like polypeptides. Preferably, naturally or non-naturally occurring 11ß-HSD1-like polypeptide variants have amino acid sequences which are at least about 44, 50, 55, 60, 65, or 70, prefer-

20   ably about 75, 80, 85, 90, 96, 96, or 98% identical to the amino acid sequence shown in SEQ ID NO:2 or a fragment thereof. Percent identity between a putative 11ß-HSD1-like polypeptide variant and an amino acid sequence of SEQ ID NO:2 is determined using the Blast2 alignment program (Blosum62, Expect 10, standard genetic codes).

25

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replace-

ments are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of an 11ß-HSD1-like polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active 11ß-HSD1-like polypeptide can readily be determined by assaying for conversion of cortisone to cortisol, as described for example, in the specific Examples, below.

Fusion Proteins

Fusion proteins are useful for generating antibodies against 11ß-HSD1-like polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of an 11ß-HSD1-like polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

An 11ß-HSD1-like polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 14, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 125, 200, or 250 contiguous amino acids of SEQ ID NO:2 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length 11ß-HSD1-like enzyme protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include ß-galactosidase, ß-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horserad-

5   ish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thiore-doxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding do-

10   main fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the 11ß-HSD1-like polypeptide-encoding sequence and the heterologous protein sequence, so that the 11ß-HSD1-like polypeptide can be cleaved and purified away from the heterologous moiety.

15

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which

20   comprises coding sequences selected from SEQ ID NOS: 1, 4, 5, or 6 in proper read-ing frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Bio-

25   technology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human 11ß-HSD1-like polypeptide can be obtained using 11ß-HSD1-like polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of 11ß-HSD1-like polypeptide, and expressing the cDNAs as is known in the art.

Polynucleotides

Coding sequence for human 11ß-HSD1-like enzyme Accession No. are found in human clones identified by GenBank Accession Nos. AW163684 (SEQ ID NO:1), AW162782 (SEQ ID NO:4), AI554725 (SEQ ID NO: 5), and AI436330 (SEQ ID NO: 6).

An 11ß-HSD1-like polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for an 11ß-HSD1-like polypeptide. Degenerate nucleotide sequences encoding human 11ß-HSD1-like polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NO:1 also are 11ß-HSD1-like polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of 11ß-HSD1-like polynucleotides which encode biologically active 11ß-HSD1-like polypeptides also are 11ß-HSD1-like polynucleotides.

Identification of Polynucleotide Variants and Homologs

Variants and homologs of the 11ß-HSD1-like polynucleotides described above also are 11ß-HSD1-like polynucleotides. Typically, homologous 11ß-HSD1-like polynu-

cleotide sequences can be identified by hybridization of candidate polynucleotides to known 11ß-HSD1-like polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50°C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the 11ß-HSD1-like polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of 11ß-HSD1-like polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5°C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol. 81*, 123 (1973). Variants of human 11ß-HSD1-like polynucleotides or 11ß-HSD1-like polynucleotides of other species can therefore be identified by hybridizing a putative homologous 11ß-HSD1-like polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO:1, 4, 5, or 6 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to 11ß-HSD1-like polynucleotides or their complements following stringent hybridization and/or wash conditions also are 11ß-HSD1-like polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

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Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20°C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a 11ß-HSD1-like polynucleo-
tide having a nucleotide sequence shown in SEQ ID NO:1, 4, 5, or 6 or the comple-
ment thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calcu-
lated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$$T_m = 81.5°C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%formamide) - 600/l),$$

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65°C, or 50% formamide, 4X SSC at 42°C, or 0.5X SSC, 0.1% SDS at 65°C. Highly stringent wash conditions include, for example, 0.2X SSC at 65°C.

Preparation of Polynucleotides

An 11ß-HSD1-like polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by
a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated 11ß-HSD1-like polynucleotides. For example, restriction enzymes
and probes can be used to isolate polynucleotide fragments which comprises 11□-
HSD1-like enzyme nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

11ß-HSD1-like enzyme cDNA molecules can be made with standard molecular bio-
logy techniques, using 11ß-HSD1-like enzyme mRNA as a template. 11ß-HSD1-like

enzyme cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize 11ß-HSD1-like polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode an 11ß-HSD1-like polypeptide having an amino acid sequence shown in SEQ ID NO:2 or a biologically active variant thereof.

Extending Polynucleotides

The partial sequences disclosed herein can be used to identify the corresponding full length gene from which they were derived. The partial sequences can be nick-translated or end-labeled with ^{32}P using polynucleotide kinase using labeling methods known to those with skill in the art (BASIC METHODS IN MOLECULAR BIOLOGY, Davis *et al.*, eds., Elsevier Press, N.Y., 1986). A lambda library prepared from human tissue can be directly screened with the labeled sequences of interest or the library can be converted *en masse* to pBluescript (Stratagene Cloning Systems, La Jolla, Calif. 92037) to facilitate bacterial colony screening (see Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press (1989, pg. 1.20).

Both methods are well known in the art. Briefly, filters with bacterial colonies containing the library in pBluescript or bacterial lawns containing lambda plaques are denatured, and the DNA is fixed to the filters. The filters are hybridized with the labeled probe using hybridization conditions described by Davis *et al.*, 1986. The partial sequences, cloned into lambda or pBluescript, can be used as positive controls

to assess background binding and to adjust the hybridization and washing stringencies necessary for accurate clone identification. The resulting autoradiograms are compared to duplicate plates of colonies or plaques; each exposed spot corresponds to a positive colony or plaque. The colonies or plaques are selected, expanded and the DNA is isolated from the colonies for further analysis and sequencing.

Positive cDNA clones are analyzed to determine the amount of additional sequence they contain using PCR with one primer from the partial sequence and the other primer from the vector. Clones with a larger vector-insert PCR product than the original partial sequence are analyzed by restriction digestion and DNA sequencing to determine whether they contain an insert of the same size or similar as the mRNA size determined from Northern blot analysis.

Once one or more overlapping cDNA clones are identified, the complete sequence of the clones can be determined. The cDNA sequence is determined from sequence analysis of multiple, overlapping clones (usually three to five overlapping sequences at each nucleotide position), resulting in a highly accurate final sequence.

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al., Nucleic Acids Res. 16*, 8186, 1988). Primers

can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation,

four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.*, GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining Polypeptides

11ß-HSD1-like polypeptides can be obtained, for example, by purification from human cells, by expression of 11ß-HSD1-like polynucleotides, or by direct chemical synthesis.

Protein Purification

11ß-HSD1-like polypeptides can be purified from any cell which expresses the enzyme, including host cells which have been transfected with 11ß-HSD1-like enzyme expression constructs. Based on the site of expression of the human sequences with GenBank Accession Nos. AW163634, AW162782, AI554725, and AI436330, it is expected that the 11ß-HSD1-like polypeptides are expressed in the fetal brain, the frontal lobe of the adult brain, and in anaplastic oligodendroglioma. A purified 11ß-HSD1-like polypeptide is separated from other compounds which normally associate with the 11ß-HSD1-like polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified 11ß-HSD1-like polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be

assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Expression of Polynucleotides

5

To express an 11ß-HSD1-like polynucleotide, the polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences

10 encoding 11ß-HSD1-like polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

15

A variety of expression vector/host systems can be utilized to contain and express sequences encoding an 11ß-HSD1-like polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression

20 vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

25 The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including consti-

30 tutive and inducible promoters, can be used. For example, when cloning in bacterial

systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a 11ß-HSD1-like polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the 11ß-HSD1-like polypeptide. For example, when a large quantity of an 11ß-HSD1-like polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the 11ß-HSD1-like polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of ß-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

- 20 -

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544,
5   1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding 11ß-HSD1-
10   like polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J. 6*, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*,
15   1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill,
20   New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express an 11ß-HSD1-like polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells
25   or in *Trichoplusia* larvae. Sequences encoding 11ß-HSD1-like polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of 11ß-HSD1-like enzyme coding sequences will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to

infect *S. frugiperda cells* or *Trichoplusia* larvae in which 11ß-HSD1-like polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-3227, 1994).

Mammalian Expression Systems

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A number of viral-based expression systems can be used to express 11ß-HSD1-like polypeptides in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding 11ß-HSD1-like polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing an 11ß-HSD1-like polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. 81*, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

15

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

20

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding 11ß-HSD1-like polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding an 11ß-HSD1-like polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The effi-

ciency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ.* 20, 125-162, 1994).

5   *Host Cells*

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed 11ß-HSD1-like polypeptide in the desired fashion.   Such modifications of the polypeptide include, but are not limited to, acet-
10   ylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function.   Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are
15   available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant
20   proteins.   For example, cell lines which stably express 11ß-HSD1-like polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector.   Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a
25   selective medium.   The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced 11ß-HSD1-like enzyme sequences.   Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.   See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

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Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 817-23, 1980) genes which can be employed in *tk⁻* or *aprt⁻* cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, □-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121-131, 1995).

Detecting Expression

Although the presence of marker gene expression suggests that the 11ß-HSD1-like polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding an 11ß-HSD1-like polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode an 11ß-HSD1-like polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding an 11ß-HSD1-like polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the 11ß-HSD1-like polynucleotide.

Alternatively, host cells which contain an 11ß-HSD1-like polynucleotide and which express an 11ß-HSD1-like polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay tech-
5   niques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a poly-nucleotide sequence encoding an 11ß-HSD1-like polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding an 11ß-HSD1-like polypeptide. Nucleic acid
10   amplification-based assays involve the use of oligonucleotides selected from se-quences encoding an 11ß-HSD1-like polypeptide to detect transformants which con-tain an 11ß-HSD1-like polynucleotide.

A variety of protocols for detecting and measuring the expression of an 11ß-HSD1-
15   like polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on an 11ß-HSD1-like polypeptide can be
20   used, or a competitive binding assay can be employed. These and other assays are described in Hampton et al., SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox et al., J. Exp. Med. 158, 1211-1216, 1983).

25   A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for produc-ing labeled hybridization or PCR probes for detecting sequences related to polynu-cleotides encoding 11ß-HSD1-like polypeptides include oligolabeling, nick transla-tion, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively,
30   sequences encoding an 11ß-HSD1-like polypeptide can be cloned into a vector for the

production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham

5   Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

10   *Expression and Purification of Polypeptides*

Host cells transformed with nucleotide sequences encoding an 11ß-HSD1-like polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be

15   secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode 11ß-HSD1-like polypeptides can be designed to contain signal sequences which direct secretion of soluble 11ß-HSD1-like polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane

20   insertion of membrane-bound 11ß-HSD1-like polypeptide.

As discussed above, other constructions can be used to join a sequence encoding an 11ß-HSD1-like polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating

25   domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor Xa

30   or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the

11□-HSD1-like polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing an 11ß-HSD1-like polypeptide and 6 histidine residues preceding a thioredoxin or an entero-kinase cleavage site. The histidine residues facilitate purification by IMAC (immobi-lized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the 11ß-HSD1-like polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993.

Chemical Synthesis

Sequences encoding an 11ß-HSD1-like polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, an 11ß-HSD1-like polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be per-formed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of 11ß-HSD1-like polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high per-formance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The compo-sition of a synthetic 11□-HSD1-like polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the 11□-HSD1-like polypep-tide can be altered during direct synthesis and/or combined using chemical methods

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with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered Polypeptides

5

As will be understood by those of skill in the art, it may be advantageous to produce 11ß-HSD1-like polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce

10 an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter 11ß-HSD1-like polypeptide-encoding sequences for a variety

15 of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation

20 patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

25 Any type of antibody known in the art can be generated to bind specifically to an epitope of an 11ß-HSD1-like polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, F(ab')₂, and Fv, which are capable of binding an epitope of an 11ß-HSD1-like polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. How-

ever, epitopes which involve non-contiguous amino acids may require more, e.g., at least 15, 25, or 50 amino acids.

5　　An antibody which specifically binds to an epitope of an 11ß-HSD1-like polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such

10　　immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

Typically, an antibody which specifically binds to an 11ß-HSD1-like polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal

15　　provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to 11ß-HSD1-like polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a 11ß-HSD1-like polypeptide from solution.

20　　11ß-HSD1-like polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, an 11ß-HSD1-like polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological re-

25　　sponse. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (e.g., aluminum hydroxide), and surface active substances (e.g. lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially useful.

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Monoclonal antibodies which specifically bind to an 11ß-HSD1-like polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature 256*, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851-6855, 1984; Neuberger *et al.*, *Nature 312*, 604-608, 1984; Takeda *et al.*, *Nature 314*, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a 11ß-HSD1-like polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to 11ß-HSD1-like polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from ran-

dom combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91).

Antibodies which specifically bind to 11ß-HSD1-like polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-3837, 1989; Winter *et al.*, *Nature 349*, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which an 11ß-HSD1-like polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of 11ß-HSD1-like enzyme gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol. Biol. 20*, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev. 90*, 543-583, 1990.

Modifications of 11ß-HSD1-like enzyme gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the 11ß-HSD1-like enzyme gene. Oligonucleotides derived from the

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transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (e.g., Gee et al., in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of an 11ß-HSD1-like polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to an 11ß-HSD1-like polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent 11ß-HSD1-like enzyme nucleotides, can provide sufficient targeting specificity for 11ß-HSD1-like enzyme mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular 11ß-HSD1-like polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to an 11ß-HSD1-like polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in

which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.,* Agrawal *et al., Trends Biotechnol. 10,* 152-158, 1992; Uhlmann *et al., Chem. Rev. 90,* 543-584, 1990; Uhlmann *et al., Tetrahedron. Lett. 215,* 3539-3542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.,* Cech, *Science 236,* 1532-1539; 1987; Cech, *Ann. Rev. Biochem. 59,* 543-568; 1990, Cech, *Curr. Opin. Struct. Biol. 2,* 605-609; 1992, Couture & Stinchcomb, *Trends Genet. 12,* 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.,* Haseloff *et al.,* U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of an 11ß-HSD1-like polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the 11ß-HSD1-like polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff *et al. Nature 334,* 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.,* EP 321,201).

Specific ribozyme cleavage sites within an 11ß-HSD1-like enzyme RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include

the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate 11ß-HSD1-

5   like enzyme RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the

10   complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA

15   construct into cells in which it is desired to decrease 11ß-HSD1-like enzyme expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element,

20   an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff et al., U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of

25   a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

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Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human 11 beta-hydroxysteroid dehydrogenase 1-like enzyme. Such genes may
5   represent genes which are differentially expressed in disorders including, but not limited to,CNS disorders, osteoporosis, liver disease, obesity, blood pressure or fetal development abnormalities, and diabetes. Further, such genes may represent genes which are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally
10   modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human 11 beta-hydroxysteroid dehydrogenase 1-like gene or gene product may itself be tested for differential expression.

15
The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quanti-
20   tative RT (reverse transcriptase), PCR, and Northern analysis.

Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is isolated
25   from tissues of interest. For example, RNA samples are obtained from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique which does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.,* ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York,
30   1987-1993. Large numbers of tissue samples may readily be processed using tech-

niques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

Transcripts within the collected RNA samples which represent RNA produced by
5   differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 85, 208-12, 1988), subtractive hybridization (Hedrick *et al.*, *Nature* 308, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 2825, 1984), and, preferably, differential display (Liang & Pardee, *Science* 257, 967-71, 1992; U.S. Patent
10   5,262,311).

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human 11 beta-hydroxysteroid dehydrogenase 1-like enzyme. For example, treatment may include a modulation of expression of the
15   differentially expressed genes and/or the gene encoding the human 11 beta-hydroxy-steroid dehydrogenase 1-like enzyme. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human 11 beta-hydroxysteroid dehydrogenase 1-like gene or gene product are up-regulated or down-regulated.
20

Screening Methods

The invention provides assays for screening test compounds which bind to or modu-late the activity of an 11ß-HSD1-like polypeptide or an 11ß-HSD1-like polynucleo-
25   tide. A test compound preferably binds to an 11ß-HSD1-like polypeptide or poly-nucleotide. More preferably, a test compound decreases or increases 11ß-HSD1 ac-tivity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The com-

5   pounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid

10   phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to poly-peptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anti-*

15   *cancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678,

20   1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J. Med. Chem. 37*, 1233, 1994). Libraries of compounds can be presented in solution (*see, e.g.*, Houghten, *BioTechniques 13*, 412-421, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-556, 1993), bacteria or spores

25   (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad Sci. U.S.A. 89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

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High Throughput Screening

Test compounds can be screened for the ability to bind to 11ß-HSD1-like polypeptides or polynucleotides or to affect 11ß-HSD1-like enzyme activity or 11ß-HSD1-like
5   enzyme gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 µl. In addition to the plates, many instru-
10   ments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a
15   simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al., Proc. Natl. Acad. Sci. U.S.A. 19,* 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark
20   pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at
25   the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially

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released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2*, 57-63
5   (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more
10   assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

15   *Binding Assays*

For binding assays, the test compound is preferably a small molecule which binds to and occupies, for example, the ATP/GTP binding site of the enzyme of the active site of the 11ß-HSD1-like polypeptide, such that normal biological activity is prevented.
20   Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules.

In binding assays, either the test compound or the 11ß-HSD1-like polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or
25   enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the 11ß-HSD1-like polypeptide can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

30

Alternatively, binding of a test compound to an 11ß-HSD1-like polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a 11ß-HSD1-like polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and an 11ß-HSD1-like polypeptide (McConnell *et al.*, *Science 257*, 1906-1912, 1992).

Determining the ability of a test compound to bind to an 11ß-HSD1-like polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, an 11ß-HSD1-like polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223-232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al.*, *BioTechniques 14*, 920-924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-1696, 1993; and Brent WO94/10300), to identify other proteins which bind to or interact with the 11ß-HSD1-like polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding an 11ß-HSD1-like polypeptide can be fused to a polynucleotide encoding

the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to

5   form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be iso-

10   lated and used to obtain the DNA sequence encoding the protein which interacts with the 11ß-HSD1-like polypeptide.

It may be desirable to immobilize either the 11ß-HSD1-like polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms

15   of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the 11ß-HSD1-like polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass

20   beads). Any method known in the art can be used to attach the 11ß-HSD1-like polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array,

25   so that the location of individual test compounds can be tracked. Binding of a test compound to a 11□-HSD1-like polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the 11ß-HSD1-like polypeptide is a fusion protein comprising a domain that allows the 11ß-HSD1-like polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed 11ß-HSD1-like polypeptide; the mixture is then incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either an 11ß-HSD1-like polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated 11ß-HSD1-like polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to an 11ß-HSD1-like polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the ATP/GTP binding site or the active site of the 11ß-HSD1-like polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the 11ß-HSD1-like polypeptide or test compound, en-

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zyme-linked assays which rely on detecting an activity of the 11ß-HSD1-like poly-peptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a 11ß-HSD1-like polypeptide or poly-nucleotide also can be carried out in an intact cell. Any cell which comprises a 11ß-HSD1-like polypeptide or polynucleotide can be used in a cell-based assay system. A 11ß-HSD1-like polynucleotide can be naturally occurring in the cell or can be intro-duced using techniques such as those described above. Binding of the test compound to a 11ß-HSD1-like polypeptide or polynucleotide is determined as described above.

Enzyme Assays

Test compounds can be tested for the ability to increase or decrease the 11ß-HSD1 activity of a human 11ß-HSD1-like polypeptide. 11ß-HSD1 activity can be measured, for example, as described in Diaz *et al.*, *J. Neurosci.* 18:2570-2580 (1988); Rajan *et al.*, *J. Neurosci.* 16:65-70 (1996); and Lloyd-MacGilp *et al.*, *Hypertension*, 34:1123-1128 (1999).

Enzyme assays can be carried out after contacting either a purified 11ß-HSD1-like polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound which decreases a transketolase activity of an 11ß-HSD1-like poly-peptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for decreasing 11ß-HSD1-like enzyme activity. A test compound which increases a transketolase activity of a human 11ß-HSD1-like polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for increasing human 11ß-HSD1-like enzyme activity.

Gene Expression

In another embodiment, test compounds which increase or decrease 11ß-HSD1-like enzyme gene expression are identified. An 11ß-HSD1-like polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the 11ß-HSD1-like polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of 11ß-HSD1-like enzyme mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of an 11ß-HSD1-like polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into an 11ß-HSD1-like polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses an 11ß-HSD1-like polynucleotide can be used in a cell-based assay system. The 11ß-HSD1-like polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

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Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, an 11ß-HSD1-like polypeptide, 11ß-HSD1-like polynucleotide, ribozymes or antisense oligonucleotides, antibodies which specifically bind to an 11ß-HSD1-like polypeptide, or mimetics, agonists, antagonists, or inhibitors of an 11ß-HSD1-like polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and

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processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxy-propylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyeth-ylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspen-

sions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

The activity of human 11ß-HSD1-like enzyme can be regulated to treat diseases such as CNS diseases, osteoporosis, liver disease, and obesity, as well as blood pressure abnormalities, abnormalities of fetal development, and diabetes.

CNS Disorders

CNS disorders which can be treated include brain injuries, cerebrovascular diseases
5 and their consequences, Parkinson's disease, corticobasal degeneration, motor neuron
disease, dementia, including ALS, multiple sclerosis, traumatic brain injury, stroke,
post-stroke, post-traumatic brain injury, and small-vessel cerebrovascular disease.
Dementias, such as Alzheimer's disease, vascular dementia, dementia with Lewy
bodies, frontotemporal dementia and Parkinsonism linked to chromosome 17, fronto-
10 temporal dementias, including Pick's disease, progressive nuclear palsy, corticobasal
degeneration, Huntington's disease, thalamic degeneration, Creutzfeld-Jakob demen-
tia, HIV dementia, schizophrenia with dementia, and Korsakoff's psychosis also can
be treated. Similarly, it is possible to treat cognitive-related disorders, such as mild
cognitive impairment, age-associated memory impairment, age-related cognitive
15 decline, vascular cognitive impairment, attention deficit disorders, attention deficit
hyperactivity disorders, and memory disturbances in children with learning disabili-
ties, by regulating the activity of human matriptase-like protein.

Osteoporosis. Osteoporosis is a disease characterized by low bone mass and micro-
20 architectural deterioration of bone tissue, leading to enhanced bone fragility and a
consequent increase in fracture risk. It is the most common human metabolic bone
disorder. Established osteoporosis includes the presence of fractures.

Bone turnover occurs by the action of two major effector cell types within bone: the
25 osteoclast, which is responsible for bone resorption, and the osteoblast, which synthe-
sizes and mineralizes bone matrix. The actions of osteoclasts and osteoblasts are
highly coordinated. Osteoclast precursors are recruited to the site of turnover; they
differentiate and fuse to form mature osteoclasts which then resorb bone. Attached to
the bone surface, osteoclasts produce an acidic microenvironment in a tightly defined
30 junction between the specialized osteoclast border membrane and the bone matrix,

thus allowing the localized solubilization of bone matrix. This in turn facilitates the proteolysis of demineralized bone collagen. Matrix degradation is thought to release matrix-associated growth factor and cytokines, which recruit osteoblasts in a temporally and spatially controlled fashion. Osteoblasts synthesize and secrete new bone

5   matrix proteins, and subsequently mineralize this new matrix. In the normal skeleton this is a physiological process which does not result in a net change in bone mass. In pathological states, such as osteoporosis, the balance between resorption and formation is altered such that bone loss occurs. See WO 99/45923.

10   The osteoclast itself is the direct or indirect target of all currently available osteoporosis agents with the possible exception of fluoride. Antiresorptive therapy prevents further bone loss in treated individuals. Osteoblasts are derived from multipotent stem cells which reside in bone marrow and also gives rise to adipocytes, chondrocytes, fibroblasts, and muscle cells. Selective enhancement of osteoblast activity is a highly

15   desirable goal for osteoporosis therapy, because it would result in an increase in bone mass rather than a prevention of further bone loss. An effective anabolic therapy would be expected to lead to a significantly greater reduction in fracture risk than currently available treatments.

20   The agonists or antagonists to the newly discovered polypeptides may act as antiresorptives by directly altering the osteoclast differentiation, osteoclast adhesion to the bone matrix, or osteoclast function of degrading the bone matrix. The agonists or antagonists could indirectly alter the osteoclast function by interfering in the synthesis and/or modification of effector molecules of osteoclast differentiation or function such

25   as cytokines, peptide or steroid hormones, proteases, etc.

The agonists or antagonists to the newly discovered polypeptides may act as anabolics by directly enhancing the osteoblast differentiation and /or its bone matrix forming function. The agonists or antagonists could also indirectly alter the osteoblast function

by enhancing the synthesis of growth factors, peptide or steroid hormones, or decreasing the synthesis of inhibitory molecules.

The agonists and antagonists may be used to mimic, augment, or inhibit the action of the newly discovered polypeptides, which may be useful to treat osteoporosis, Paget's disease, degradation of bone implants, and, particularly, dental implants.

Liver disease. All chronic liver diseases cause the development of fibrosis in the liver. Fibrosis is a programmed uniform wound healing response. The most important chronic liver diseases are viral hepatitis B and C and alcohol-induced liver disease. Between 10 and 30% of patients affected develop cirrhosis as a late complication. Liver cirrhosis has a 5-year survival rate of 50%. Deaths from liver cirrhosis occurs at an average age of only 60 years. It is the ninth largest cause of death in the USA.

Toxic damage or injury caused by foreign proteins causes the deposition of extracellular matrix such as collagen, fibronectin and laminin. The common mechanism is the activation and transformation of vitamin-A storing hepatic stellate cells (Ito cells) into matrix producing myofibroblasts. These proliferate and fill the extracellular Space of Disse with extracellular matrix. This process contains para- and autocrine activation steps, which cause it to become auto-perpetuated if the process of injury is sustained for a long period of time. It causes a slowly progressing shunt, which reduces the perfusion of the liver with portal and arterial blood. This results in a loss of liver function. Build-up of an increased diffusion barrier by a loss of fenestration in the sinusoidal endothelium. This corroborates the loss in function. Portal hypertension with the frequent complication of esophagal bleeding. Causes of liver disease death include hepatic coma (30%), esophagal bleeding (30%), and primary hepatic carcinoma (30%).

Indications for which regulation of this protein may be useful are liver fibrosis and cirrhosis caused by chronic degenerative diseases of the liver such as viral hepatitis,

alcohol hepatitis, autoimmune hepatitis, primary biliary cirrhosis, cystic fibrosis, hemochromatosis, Wilson's disease, non-alcoholic steato-hepatitis, and others. Possible other indications are the treatment of systemic sclerosis, pulmonary fibrosis, pancreatic fibrosis, myocardial fibrosis, and prostatic fibrosis.

5

Obesity. Obesity and overweight are defined as an excess of body fat relative to lean body mass. An increase in caloric intake or a decrease in energy expenditure or both can bring about this imbalance leading to surplus energy being stored as fat. Obesity is associated with important medical morbidities and an increase in mortality. The

10 causes of obesity are poorly understood and may be due to genetic factors, environmental factors or a combination of the two to cause a positive energy balance. In contrast, anorexia and cachexia are characterized by an imbalance in energy intake versus energy expenditure leading to a negative energy balance and weight loss. Agents that either increase energy expenditure and/or decrease energy intake, absorp-

15 tion or storage would be useful for treating obesity, overweight, and associated comorbidities. Agents that either increase energy intake and/or decrease energy expenditure or increase the amount of lean tissue would be useful for treating cachexia, anorexia and wasting disorders.

20 This gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity, overweight, anorexia, cachexia, wasting disorders, appetite suppression, appetite enhancement, increases or decreases in satiety, modulation of body weight, and/or other eating disorders such as bulimia. Also this gene, translated proteins and agents which modulate this gene or portions of

25 the gene or its products are useful for treating obesity/overweight-associated comorbidities including hypertension, type 2 diabetes, coronary artery disease, hyperlipidemia, stroke, gallbladder disease, gout, osteoarthritis, sleep apnea and respiratory problems, some types of cancer including endometrial, breast, prostate, and colon cancer, thrombolic disease, polycystic ovarian syndrome, reduced fertility, complica-

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tions of pregnancy, menstrual irregularities, hirsutism, stress incontinence, and depression.

Diabetes

Diabetes mellitus is a common metabolic disorder characterized by an abnormal elevation in blood glucose, alterations in lipids and abnormalities (complications) in the cardiovascular system, eye, kidney and nervous system. Diabetes is divided into two separate diseases: type 1 diabetes (juvenile onset), which results from a loss of cells which make and secrete insulin, and type 2 diabetes (adult onset), which is caused by a defect in insulin secretion and a defect in insulin action.

Type 1 diabetes is initiated by an autoimmune reaction that attacks the insulin secreting cells (beta cells) in the pancreatic islets. Agents that prevent this reaction from occurring or that stop the reaction before destruction of the beta cells has been accomplished are potential therapies for this disease. Other agents that induce beta cell proliferation and regeneration also are potential therapies.

Type II diabetes is the most common of the two diabetic conditions (6% of the population). The defect in insulin secretion is an important cause of the diabetic condition and results from an inability of the beta cell to properlydetect and respond to rises in blood glucose levels with insulin release. Therapies that increase the response by the beta cell to glucose would offer an important new treatment for this disease.

The defect in insulin action in Type II diabetic subjects is another target for therapeutic intervention. Agents that increase the activity of the insulinreceptor in muscle, liver, and fat will cause a decrease in blood glucose and anormalization of plasma lipids. The receptor activity can be increased by agents that directly stimulate the receptor or that increase the intracellular signals from the receptor. Other therapies can directly activate the cellularend process, *i.e.* glucose transport or various enzyme

systems, to generate aninsulin-like effect and therefore a produce beneficial outcome. Becauseoverweight subjects have a greater susceptibility to Type II diabetes, any agent that reduces body weight is a possible therapy.

5   Both Type I and Type diabetes can be treated with agents that mimic insulinaction or that treat diabetic complications by reducing blood glucose levels. Likewise, agents that reduces new blood vessel growth can be used to treat the eye complications that develop in both diseases.

10   This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or an 11ß-HSD1-like polypep-
15   tide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described
20   herein.

A reagent which affects 11ß-HSD1-like enzyme activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce 11ß-HSD1-like enzyme activity. The reagent preferably binds to an expression product of a human 11ß-HSD1-like enzyme
25   gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

30

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleo-

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tide is combined with about 8 nmol of liposomes, more preferably from about 0.5 µg to about 5 µg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 µg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al., J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al., Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al., J. Biol. Chem. 266*, 338-42 (1991).

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases 11ß-HSD1-like enzyme activity relative to the 11ß-HSD1-like enzyme activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals.

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The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established

techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 µg to about 50 µg/kg, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of an 11ß-HSD1-like enzyme gene or the activity of an 11ß-HSD1-like polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a 11ß-HSD1-like enzyme gene or the activity of an 11ß-HSD1-like polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to 11ß-HSD1-like enzyme-specific mRNA, quantitative RT-PCR, immunologic detection of an 11ß-HSD1-like polypeptide, or measurement of 11ß-HSD1-like enzyme activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be

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made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus

5   reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

10

Diagnostic Methods

Human 11ß-HSD1-like enzyme also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the

15   presence of mutations in the nucleic acid sequences which encode the enzyme. For example, differences can be determined between the cDNA or genomic sequence encoding 11ß-HSD1-like enzyme in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the dis-

20   ease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this

25   method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

30

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be

5   distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers et al., Science 230, 1242, 1985). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method

10   (e.g., Cotton et al., Proc. Natl. Acad. Sci. USA 85, 4397-4401, 1985). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected

15   by in situ analysis.

Altered levels of an 11ß-HSD1-like enzyme also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art

20   and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention.

25   A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of 11□-HSD1-like enzyme activity

The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4
5   and the expression vector pCEV4-11ß-HSD1-like enzyme polypeptide obtained is
transfected into human embryonic kidney 293 cells. From these cells extracts are
obtained and 11ß-HSD1 activity is determined by measuring the conversion of 12
mmol/L H-corticosterone (1,2,6,7 H-corticosterone, specific activity 86 Ci/mmol;
Amersham Life Sciences Ltd.) to radioactive 11-dehydrocorticosterone in the presence
10   of a range of concentrations of cold corticosterone (0 to 10 µmol/L) and either NADP
or NAD as cofactor (0.2 mmol/L). Steroids are extracted into ethyl acetate and are
separated by high-performance liquid chromatography. Maximal velocity (V_{max}) and
K_m values are estimated using the percent conversion of H-corticosterone to 11-dehy-
drocorticosterone with the various concentrations of cold corticosterone. It is shown
15   that SEQ ID NO: 2 has a 11ß-HSD1-like enzyme activity.

EXAMPLE 2

Expression of recombinant human 11□-HSD1-like enzyme

20   The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to
produce large quantities of recombinant human 11ß-HSD1 polypeptides in yeast. The
11□-HSD1-like enzyme-encoding DNA sequence comprises SEQ ID NO:1. Before
insertion into vector pPICZB, the DNA sequence is modified by well known methods
in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an
25   enterokinase cleavage site, a His6 reporter tag and a termination codon. Moreover, at
both termini recognition sequences for restriction endonucleases are added and after
digestion of the multiple cloning site of pPICZ B with the corresponding restriction
enzymes the modified DNA sequence is ligated into pPICZB. This expression vector
is designed for inducible expression in *Pichia pastoris*, driven by a yeast promoter.
30   The resulting pPICZ/md-His6 vector is used to transform the yeast

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The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with

5   buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human 11☐-HSD1-like polypeptide is obtained.

10   **EXAMPLE 3**

Determination of 11β-HSD1 activity in a kidney cell; Identification of compounds that alter 11β-HSD1 activity.

11β-HSD1 activity is measured essentially as in Lloyd-MacGilp, *supra*. Kidneys are

15   sectioned (50 μm) in ice-cold Krebs-Ringer solution (0.1 mol/L NaCl, 2.5 mmol/L KCl, 2.5 mmol/L CaCl$_2$, 1.2 mmol/L KH$_2$PO$_3$, 1.2 mmol/L MgSO$_4$, and 25 mmol/L NaHCO$_3$)., and cortical tissues are dissected from inner medullary tissue. Each region is processed separately. After homogenization in 4 volumes of Ringer's solution, the homogenates are centrifuged at 16,0000 x g for 20 minutes, followed by centrifugation

20   of the supernatant at for one hour at 10,600 x g and 4 °C to prepare microsomal fractions.

Aliquots of the reaction are contacted with test compounds from a small molecule library. Control samples are incubated in the absence of test compounds. After incu-

25   bation for ten minutes at 37 °C, 2 ml ethyl acetate is added to terminate the reactions.

Dehydrogenase activity in microsomes is determined by measuring the conversion of 12 mmol/L H-corticosterone (1,2,6,7 H-corticosterone, specific activity 86 Ci/mmol; Amersham Life Sciences Ltd.) to radioactive 11-dehydrocorticosterone in the presence

30   of a range of concentrations of cold corticosterone (0 to 10 ☐mol/L) and either NADP

or NAD as cofactor (0.2 mmol/L). Steroids are extracted into ethyl acetate and are separated by high-performance liquid chromatography. Maximal velocity (V_{max}) and K_m values are estimated using the percent conversion of H-corticosterone to 11-dehydrocorticosterone with the various concentrations of cold corticosterone. A test com-

5   pound which increases or decreases V_{max} or K_m values by 20% is identified as a potential therapeutic agent for regulating 11ß-HSD1-like enzyme.

EXAMPLE 4

Identification of a test compound which decreases 11β-HSD1-like enzyme gene ex-
10   *pression*

A test compound is administered to a culture of human cells transfected with an 11ß-HSD1-like enzyme expression construct and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells which have not been transfected is incubated for
15   the same time without the test compound to provide a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ^{32}P-labeled 11ß-HSD1-like enzyme-specific probe at 65°C in Express-
20   hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO:1. A test compound which decreases the 11ß-HSD1-like enzyme-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of 11ß-HSD1-like enzyme gene expression.

25   **EXAMPLE 5**

Treatment of a central nervous system disorder with a reagent which specifically binds to an 11β-HSD1 gene product

Synthesis of antisense 11ß-HSD1 oligonucleotides comprising at least 11 contiguous
30   nucleotides selected from the complement of SEQ ID NO:1 is performed on a Phar-

macia Gene Assembler series synthesizer using the phosphoramidite procedure. Uhl-mann *et al., supra.* Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the *Limulus* Amebocyte Assay. Bang, *Biol. Bull. (Woods Hole, Mass.) 105,* 361-362 (1953).

An aqueous composition containing the antisense oligonucleotides at a concentration of 0.1-100 µM is administered by injection to a patient suffering from a CNS disorder. The severity of the disorder is thereby decreased.

CLAIMS

1.   An isolated polynucleotide encoding a 11β-HSD1-like enzyme polypeptide and being selected from the group consisting of:

   a)   a polynucleotide encoding a 11β-HSD1-like enzyme polypeptide comprising an amino acid sequence selected form the group consisting of:
   amino acid sequences which are at least about 50% identical to
   the amino acid sequence shown in SEQ ID NO: 2; and
   the amino acid sequence shown in SEQ ID NO: 2.

   b)   a polynucleotide comprising the sequence of SEQ ID NO: 1;

   c)   a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

   d)   a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

   e)   a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a to (d).

2.   An expression vector containing any polynucleotide of claim 1.

3.   A host cell containing the expression vector of claim 2.

4.   A substantially purified 11β-HSD1-like enzyme polypeptide encoded by a polynucleotide of claim 1.

5.   A method for producing a 11β-HSD1-like enzyme polypeptide, wherein the method comprises the following steps:

   a)   culturing the host cell of claim 3 under conditions suitable for the expression of the 11β-HSD1-like enzyme polypeptide; and

b)   recovering the 11β-HSD1-like enzyme polypeptide from the host cell culture.

6.   A method for detection of a polynucleotide encoding a 11β-HSD1-like enzyme polypeptide in a biological sample comprising the following steps:

a)   hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b)   detecting said hybridization complex.

7.   The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8.   A method for the detection of a polynucleotide of claim 1 or a 11β-HSD1-like enzyme polypeptide of claim 4 comprising the steps of:

contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the 11β-HSD1-like enzyme polypeptide.

9.   A diagnostic kit for conducting the method of any one of claims 6 to 8.

10.   A method of screening for agents which decrease the activity of a 11β-HSD1-like enzyme, comprising the steps of:

contacting a test compound with any 11β-HSD1-like enzyme polypeptide encoded by any polynucleotide of claim1;

detecting binding of the test compound to the 11β-HSD1-like enzyme polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a 11β-HSD1-like enzyme.

11.   A method of screening for agents which regulate the activity of a 11β-HSD1-like enzyme, comprising the steps of:

contacting a test compound with a 11β-HSD1-like enzyme polypeptide encoded by any polynucleotide of claim 1; and

detecting a 11βb-HSD1-like enzyme activity of the polypeptide, wherein a test compound which increases the 11β-HSD1-like enzyme activity is identified as

5   a potential therapeutic agent for increasing the activity of the 11β-HSD1-like enzyme, and wherein a test compound which decreases the 11β-HSD1-like enzyme activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the 11β-HSD1-like enzyme.

10   12.   A method of screening for agents which decrease the activity of a 11β-HSD1-like enzyme, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent

15   for decreasing the activity of 11β-HSD1-like enzyme.

13.   A method of reducing the activity of 11β-HSD1-like enzyme, comprising the steps of:

contacting a cell with a reagent which specifically binds to any polynucleotide

20   of claim 1 or any 11β-HSD1-like enzyme polypeptide of claim 4, whereby the activity of 11β-HSD1-like enzyme is reduced.

14.   A reagent that modulates the activity of a 11β-HSD1-like enzyme polypeptide or a polynucleotide wherein said reagent is identified by the method of any of

25   the claim 10 to 12.

15.   A pharmaceutical composition, comprising:

the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16.   Use of the pharmaceutical composition of claim 15 for modulating the activity
        of a 11β-HSD1-like enzyme in a disease.

5   17.   Use of claim 16 wherein the disease is CNS disorder, osteoporosis, liver dis-
        ease, obesity, blood pressure or fetal development abnormality and diabetes.

18.   A cDNA encoding a polypeptide comprising the amino acid sequence shown
        in SEQ ID NO:2.

10

19.   The cDNA of claim 18 which comprises SEQ ID NO:1.

20.   The cDNA of claim 18 which consists of SEQ ID NO:1.

15   21.   An expression vector comprising a polynucleotide which encodes a polypep-
        tide comprising the amino acid sequence shown in SEQ ID NO:2.

22.   The expression vector of claim 21 wherein the polynucleotide consists of SEQ
        ID NO:1.

20

23.   A host cell comprising an expression vector which encodes a polypeptide
        comprising the amino acid sequence shown in SEQ ID NO:2.

24.   The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO:1.

25

25.   A purified polypeptide comprising the amino acid sequence shown in SEQ ID
        NO:2.

26.   The purified polypeptide of claim 25 which consists of the amino acid se-
30   quence shown in SEQ ID NO:2.

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27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO:2.

5   28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:
culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and
isolating the polypeptide.

10

29. The method of claim 28 wherein the expression vector comprises SEQ ID NO:1.

30. A method of detecting a coding sequence for a polypeptide comprising the
15   amino acid sequence shown in SEQ ID NO:2, comprising the steps of:
hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and
detecting the hybridization complex.

20

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

32. A kit for detecting a coding sequence for a polypeptide comprising the amino
25   acid sequence shown in SEQ ID NO:2, comprising:
a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1; and
instructions for the method of claim 30.

33. A method of detecting a polypeptide comprising the amino acid sequence
30   shown in SEQ ID NO:2, comprising the steps of:

contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and

detecting the reagent-polypeptide complex.

5   34.   The method of claim 33 wherein the reagent is an antibody.

       35.   A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising:

              an antibody which specifically binds to the polypeptide; and

10   instructions for the method of claim 33.

       36.   A method of screening for agents which can modulate the activity of a human 11β-HSD1-like enzyme, comprising the steps of:

              contacting a test compound with a polypeptide comprising an amino acid se-

15   quence selected from the group consisting of: (1) amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO:2 and (2) the amino acid sequence shown in SEQ ID NO:2; and

              detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for

20   regulating activity of the human 11β-HSD1-like enzyme.

       37.   The method of claim 36 wherein the step of contacting is in a cell.

       38.   The method of claim 36 wherein the cell is *in vitro*.

25

       39.   The method of claim 36 wherein the step of contacting is in a cell-free system.

       40.   The method of claim 36 wherein the polypeptide comprises a detectable label.

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41.   The method of claim 36 wherein the test compound comprises a detectable label.

42.   The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43.   The method of claim 36 wherein the polypeptide is bound to a solid support.

44.   The method of claim 36 wherein the test compound is bound to a solid support.

45.   A method of screening for agents which modulate an activity of a human 11β-HSD1-like enzyme, comprising the steps of:

        contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO:2 and (2) the amino acid sequence shown in SEQ ID NO:2; and

        detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human 11β-HSD1-like enzyme, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human 11β-HSD1-like enzyme.

46.   The method of claim 45 wherein the step of contacting is in a cell.

47.   The method of claim 45 wherein the cell is *in vitro*.

48.   The method of claim 45 wherein the step of contacting is in a cell-free system.

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49. A method of screening for agents which modulate an activity of a human 11β-HSD1-like enzyme, comprising the steps of:

contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO:1; and

5   detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human 11β-HSD1-like enzyme.

50. The method of claim 49 wherein the product is a polypeptide.

10

51. The method of claim 49 wherein the product is RNA.

52. A method of reducing activity of a human 11β-HSD1-like enzyme, comprising the step of:

15   contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:1, whereby the activity of a human 11β-HSD1-like enzyme is reduced.

53. The method of claim 52 wherein the product is a polypeptide.

20

54. The method of claim 53 wherein the reagent is an antibody.

55. The method of claim 52 wherein the product is RNA.

25 56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.

57. The method of claim 56 wherein the reagent is a ribozyme.

58. The method of claim 52 wherein the cell is *in vitro*.

30

59. The method of claim 52 wherein the cell is *in vivo*.

60. A pharmaceutical composition, comprising:
   a reagent which specifically binds to a polypeptide comprising the amino acid
   sequence shown in SEQ ID NO:2; and
   a pharmaceutically acceptable carrier.

61. The pharmaceutical composition of claim 60 wherein the reagent is an antibody.

62. A pharmaceutical composition, comprising:
   a reagent which specifically binds to a product of a polynucleotide comprising
   the nucleotide sequence shown in SEQ ID NO:1; and
   a pharmaceutically acceptable carrier.

63. The pharmaceutical composition of claim 62 wherein the reagent is a ribozyme.

64. The pharmaceutical composition of claim 62 wherein the reagent is an antisense oligonucleotide.

65. The pharmaceutical composition of claim 62 wherein the reagent is an antibody.

66. A pharmaceutical composition, comprising:
   an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2; and
   a pharmaceutically acceptable carrier.

67.   The pharmaceutical composition of claim 66 wherein the expression vector comprises SEQ ID NO:1.

68.   A method of treating a 11β-HSD1-like enzyme disfunction related disease, wherein the disease is selected from CNS disorder, osteoporosis, liver disease, obesity, blood pressure or fetal development abnormality, and diabetes, comprising the step of:

        administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human 11β-HSD1-like enzyme, whereby symptoms of the 11β-HSD1-like enzyme disfunction related disease are ameliorated.

69.   The method of claim 68 wherein the reagent is identified by the method of claim 36.

70.   The method of claim 68 wherein the reagent is identified by the method of claim 45.

71.   The method of claim 68 wherein the reagent is identified by the method of claim 49.

LIO 101-Foreign Countries

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Fig. 1

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LIO 101-Foreign Countries

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Fig. 2

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  5  HTEALLQKVV GNCRKLAAPR SSHRGGPGLX XGARERGAFA LDQLGGLDSS CXPHSRAMAA

     REHAALATXC SCXXLCELLH XLXASCXLDD IXASXXVXLX X
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LIO 101-Foreign Countries

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Fig. 3

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5    TMSLFHDDIH SVRRSMEVNF LSYVVLSTAA LPMLKQSNGS IAIISSMAGK MTQPLIASYS

     ASKFALDGFF STIRKEHLMT KVNVSITLCV LGFIDTETAL KETSGIILSQ AAPKQECALE

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LIO 101-Foreign Countries

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Fig. 4

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10   aaggtcttct acatcgcggc ggacatggcc tcccttgag cacccgagag cgtggtgcaa

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LIO 101-Foreign Countries

- 5/7 -

Fig. 5

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LIO 101-Foreign Countries

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Fig. 6

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10 gggtcgaagt t

LIO 101-Foreign Countries

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Fig. 7

BLASTX - Query = AW163684; Hit = swiss|P16232|DHI1_RAT

5   This hit is scoring at : 3e-11 (expectation value)

Alignment length (overlap) : 86

Identities : 43 %

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Query reading frame : +3

10 Database searched : nrdb

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H:    25   PEMLQGKKVIVTGASKGIGREMAYHLSKMGAHVVLTARSEEGLQKVVSRCLELGAASAHY
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Arg Phe Val Val Glu Ala Gly Lys Leu Leu Gly Gly Leu Asp Met Leu
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